PB86-195146

Sediment Toxicity to a Marine Infaunal Amphipod Cadmium and Its Interaction with Sewage Sludge

(U.S.) Environmental Research Lab.
Narragansett, Newport, OR

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completin

1. REPORT NO. EPA/600/J-85/382	2.
3. R PB86-195146	
4. TITLE AND SUBTITLE SEDIMENT TOXICITY TO A MARINE INFAUNAL AMPHIPOD: CADMIUM AND ITS INTERACTION WITH SEWAGE SLUDGE (Journal Version)	5. REPORT DATE 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R.C. Swartz, G.R. Ditsworth, D.W. Schults and J.O. Lamberson	8. PERFORMING ORGANIZATION REPORT NO. ERLN-N006
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S.EPA, ERLN-Newport Hatfield Marine Science Center Newport, OR 97365	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same AS Above	13. TYPE OF REPORT AND PERIOD COVERED Journal Article
	14. SPONSORING AGENCY CODE EPA/600/05

15. SUPPLEMENTARY NOTES

Marine Environmental Research, Vol.18,pp.133-153 (1985)

16. ABSTRACT

The acute toxicity of cadmium to the marine infaunal amphipod, Rhepoxynius abronius, was determined separately in sediment and seawater. Most cadmium added to test sediment was bound to particles and less than 5% was dissolved in interstitial water. The LC50 based on cadmium concentration in interstitial water was similar to the LC50 based on cadmium concentratration in seawater without sediment. Cadmium in interstitial water , rather than that bound to particles, therefore appears responsible for acute sediment toxicity to this species. The addition of small quantities of sewage sludge or an increase in the proportion of the fine fraction of sediment particles significantly reduced the toxicity of cadmium in sediment. Binding of cadmium by sediment particles may explain the presence of phoxocephalid amphipods at sites where sewage and metal pollution occur together. The cadmium sensitivity of R. abronius is similar to that of several marine invertebrates commonly used in seawater bioassays. This amphipod is a useful test species for sediment bioassays used in research, monitoring and regulatory programs.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT DISTRIBUTION UNLIMITED RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 23
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDI

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA/600/J-85/382
JOURNAL ARTICLE

Marine Environmental Research **18** (1985) 133–153

Sediment Toxicity to a Marine Infaunal Amphipod: Cadmium and its Interaction with Sewage Sludge

R. C. Swartz, G. R. Ditsworth,
D. W. Schults & J. O. Lamberson

US Environmental Protection Agency, Mark O. Hatfield Marine Science Center, Newport, Oregon 97365, USA

(Received: 26 September, 1985)

ABSTRACT

The acute toxicity of cadmium to the marine infaunal amphipod, Rhepoxynius abronius, *was determined separately in sediment and seawater. Most cadmium added to test sediment was bound to particles and less than 5% was dissolved in interstitial water. The* LC_{50} *based on cadmium concentration in interstitial water was similar to the* LC_{50} *based on cadmium concentration in seawater without sediment. Cadmium in interstitial water, rather than that bound to particles, therefore appears responsible for acute sediment toxicity to this species. The addition of small quantities of sewage sludge or an increase in the proportion of the fine fraction of sediment particles significantly reduced the toxicity of cadmium in sediment. Binding of cadmium by sediment particles may explain the presence of phoxocephalid amphipods at sites where sewage and metal pollution occur together. The cadmium sensitivity of* R. abronius *is similar to that of several marine invertebrates commonly used in seawater bioassays. This amphipod is a useful test species for sediment bioassays used in research, monitoring and regulatory programs.*

INTRODUCTION

Cadmium accumulates in the seabed around sewage outfalls and other sites of marine pollution (Helz *et al.*, 1975; Bryan, 1976; Hershelman *et al.*, 1981). Oakden *et al.* (1984*a*,*b*) have recently shown that amphipods

Marine Environ. Res. 0141-1136/86/$03·50
England, 1986. Printed in Great Britain

will avoid sediment containing cadmium at potentially lethal concentrations. However, there is little published information on the concentrations of cadmium in sediment that are toxic to marine organisms.

Since cadmium can dissolve in interstitial water and also bind to sediment particles, its relative toxicity in different sediment phases is important to pollution assessments. Comparison of the toxicity of particle-bound and interstitial cadmium has not been made directly and must be inferred from the literature on cadmium bioaccumulation. The cadmium binding capacity of estuarine sediment particles is due primarily to iron oxides, manganese oxides and organic matter (Davies-Colley *et al.*, 1984). Materials added to sediment by human activities (e.g. sewage) have different metal-binding capacities than natural sediments (Riffaldi *et al.*, 1983; Thomson *et al.*, 1984). In experiments with isolated sediment phases, the deposit-feeding clam *Macoma balthica* accumulated cadmium bound to iron oxide particles, but did not accumulate cadmium bound to organic matter or organically coated iron oxide particles (Luoma & Jenne, 1976). This suggests that cadmium bound to only certain sites on sediment particles may exert toxic effects on benthic animals. However, the validity of extrapolating results obtained with isolated sediment phases to intact sediments is uncertain (Luoma & Jenne, 1976). Other reports indicate that cadmium in sediment must partition into water before accumulation and toxicity are possible (Hardy *et al.*, 1981; Schuytema *et al.*, 1984).

Interactions between sediment organic enrichment and the toxicity of cadmium in sediment are important because high concentrations of metals often occur in organic wastes. The freshwater Asiatic clam *Corbicula fluminea* accumulated significantly greater amounts of cadmium from sediment that contained no organic matter than from sediment that contained ground oak leaves (Graney *et al.*, 1984). If organic enrichment decreases the availability and consequently the toxicity of cadmium in sediment, this interaction may explain the presence of cadmium-sensitive species in areas where both organic pollution and cadmium contamination are relatively high (Swartz *et al.*, 1985*a*).

The first objective of the present research was to determine the acute toxicity of cadmium in sediment to the phoxocephalid amphipod, *Rhepoxynius abronius*. This is an infaunal species that does not extend tubes or body parts into overlying seawater and, therefore, is exposed to ambient contaminant concentrations in interstitial water and on

sediment particles (Swartz *et al.*, 1985*b*). Four- and 10-day bioassays were conducted to estimate total cadmium concentrations in sediment that caused mortality or sublethal effects on test amphipods.

The second objective was to determine the relative contributions of interstitial and particle-bound cadmium to toxicity. The 4-day LC_{50} of cadmium in interstitial water was compared with the 4-day LC_{50} of cadmium in seawater without sediment. The similarity of these LC_{50}s would indicate that toxicity in the sediment bioassay can be attributed to the cadmium dissolved in interstitial water. Differences between these LC_{50}s would suggest that factors other than dissolved cadmium affected survival in the sediment bioassay. Toxicity of particle-bound cadmium would be evident if sediments were more toxic than predicted by cadmium concentrations in interstitial water.

The third objective was to examine the relationship between cadmium toxicity and enrichment of the native sediment of *R. abronius* by sewage sludge and the fine fraction of natural sediment. The interaction between three concentrations of cadmium and three sediment enrichments was tested in a two-way experimental design. Although these sediment enrichments were quantified by measuring organic content (volatile solids), they affected both organic and inorganic sediment characteristics.

MATERIALS AND METHODS

Collection of sediment and amphipods

The phoxocephalid amphipod bioassay for sediment toxicity was used in this research (Swartz *et al.*, 1985*b*). The infaunal phoxocephalid *R. abronius* and clean (control) sediment were collected with a small dredge from lower Yaquina Bay, Oregon (44°37′33″N, 124°02′58″W). Adult animals (~4 mm total length) were removed by gently sieving sediment through a 1·0 mm screen. Amphipods were picked from the material retained on the screen and maintained in sieved sediment until the bioassay was initiated 3–4 days later. A portion of the sediment that passed through the 1·0 mm screen was resieved through a 0·5 mm screen into 0·45-μm filtered, 25 ppt (parts per thousand) seawater. The sediment was allowed to settle for 5 h and then stored at 4°C for 2–3 days until it was used to prepare the test materials. The second sieving removed all juvenile *R. abronius* and adjusted interstitial salinity to a constant

test condition of 25 ppt. Sediment at the collection site is a well-sorted, fine sand.

Preparation of test material

Cadmium stock solutions were prepared by dissolving reagent grade cadmium chloride ($CdCl_2 . 2^1/_2 H_2O$) in 0·45 μm-filtered, 25 ppt seawater. Stock solutions were serially diluted with seawater and added to either seawater or sediment to produce the desired test concentrations of cadmium metal.

Wet sediment for the bioassay was thoroughly mixed with a plastic spoon, divided into 3·5 kg aliquots and placed in 1-gallon (3·8-liter) glass jars. Total solids of the sediment was determined to calculate the amount of cadmium chloride necessary to produce the desired concentration of Cd metal per dry weight of sediment in each jar. The Cd solution was dripped into several holes punched into the sediment in each jar at different depths. The sediment was then mixed by rolling the jars at about 15 rpm for 2 h. Sediment on the sides of the jar was scraped down and mixed with the bulk of the sediment. Jars were then stored overnight at 4°C, rolled for 2 h the next day, mixed by spoon and placed in the bioassay chambers. Amphipods were not added to the chambers until the next day, about 40 h after the cadmium solution was initially added to the sediment. Control sediment received no toxic materials but was rolled as described above.

Sewage sludge and natural sediment extracts were also mixed into sediment by the rolling procedure. The natural organic content of control sediment was increased by adding an extract of the high-organic, fine fraction of Yaquina Bay sediment. This extract was prepared by creating a suspension of control sediment, allowing it to settle for 15 s to remove coarse silts and sands, decanting the remaining suspension and allowing particles in the decanted material to settle for 12 h. This process was repeated with several lots of control sediment to produce about 1 kg (dry weight) of settled particles with a total volatile solids content of 11·6%. An appropriate proportion of the extract was then rolled into unadulterated control sediment to produce the desired increase in natural sediment organic matter. This procedure probably increased other sediment parameters, e.g. iron and manganese oxides.

Digested sewage sludge was obtained from a secondary treatment plant in the small residential community of Waldport, Oregon. No

major industries are connected to this sewer system. The sludge was dewatered by centrifugation and mixed with sediment prior to testing. Previous research showed that a Waldport sludge addition that increases the total volatile solids of Yaquina Bay sediment from our collection site by 0·25% produces no toxic effect on *R. abronius* (Swartz *et al.*, 1984). A nominal increase of 0·25% total volatile solids was therefore used to examine interactions between cadmium and sewage sludge or fine material from natural sediment.

Bioassay procedure

Bioassay chambers (1 liter glass beakers) were filled to a 2-cm depth with test sediment. A plastic disc was placed on the sediment surface to minimize disturbance as the beakers were filled to the 750 ml level with 25 ppt seawater. A separate disc was used for each test sediment. Test chambers were covered with watch glasses and aerated through a 1 ml pipette that extended no closer than 2 cm above the sediment surface. The beakers were placed in a 15°C water bath and allowed to equilibrate overnight. In experiments in which cadmium was added to sediment, cadmium was never added to seawater overlying the sediment. The bioassay procedure for experiments in which cadmium was added to seawater was identical to the sediment tests except for the absence of sediment. Five replicate bioassay chambers were prepared for all treatments in all experiments.

The bioassay was initiated by distributing 20 randomly chosen *R. abronius* to each chamber along with 200 ml of seawater. This final addition of clean seawater completed the dilution of cadmium to the desired seawater concentration in experiments without sediment. Typically, the amphipods quickly swam or sank to the bottom and burrowed into the sediment, or, in the absence of sediment, rested on the bottom with occasional attempts at swimming or burrowing. The number of amphipods that had emerged from sediment was recorded daily. After 4 or 10 days the contents of the beakers were sieved through a 0·5 mm screen and survivors counted and placed in a bowl with fresh seawater and control sediment. The number that failed to rebury after 1 h was recorded.

Analytical chemistry

Three kinds of samples were collected for cadmium analysis: (1) seawater, (2) sediment, including particles and interstitial water, and (3) interstitial

water drained from sediment. Seawater was obtained from the bioassay beakers by withdrawing a mid-water column sample into a 10 cm^3 glass syringe. The sample was then preserved for cadmium analysis by reducing its pH to <2 with concentrated HCl (Ultrex). Seawater in bioassays conducted without sediment was filtered (0·45 μm) prior to the addition of cadmium, but samples taken at the end of the bioassay were not filtered again. Sediment samples were obtained by slowly tilting a beaker and removing the seawater down to the sediment surface. The beaker was then placed in a nitrogen atmosphere in a glove bag, the sediment was mixed and a sample taken for cadmium analysis. The remaining sediment was placed in the funnel of a Millipore aseptic filtering apparatus in the glove bag and interstitial water collected by vacuum filtration through a glass-fiber prefilter and a 0·45-μm Nuclepore final filter. These filters retain a negligible quantity of dissolved cadmium. The interstitial water sample was also treated with HCl to reduce its pH to <2.

Seawater and interstitial water samples with cadmium concentrations <2 mg $liter^{-1}$ were analyzed by flame atomic absorption (Environmental Protection Agency, 1979). Samples with cadmium concentrations >2 mg $liter^{-1}$ were diluted with 25 ppt seawater before analysis. Cadmium standards were prepared in 25 ppt seawater and also acidified.

Sediment samples were dried at 105°C overnight and 2 g of the homogeneous sample were shaken with 10 ml of 4N HCl for 15 min. The extracts were filtered through a Whatman No. 40 paper filter and diluted to 25 ml with distilled water. A methods blank (extraction acid without sediment) showed negligible cadmium contamination by the extraction and filtration technique. The sediment extracts were analyzed by flame atomic absorption. Cadmium standards were prepared in distilled water. All concentrations of cadmium in sediment are expressed on a dry weight basis.

Cadmium concentrations in sediment and seawater did not change greatly during bioassays. Measured concentrations of cadmium in sediment were 99·7% $\pm$ 4·4% (mean $\pm$ 95% confidence limits) of nominal concentrations. Concentrations of cadmium in sediment at the end of the bioassays were 98·4% $\pm$ 4·3% of the concentrations at the beginning (day 0). Measured concentrations of cadmium in seawater were consistently higher (118·1% $\pm$ 2·5%) than nominal concentrations. Concentrations of cadmium in seawater were only slightly lower (94·8% $\pm$ 4·1%) at the end of the bioassay than at the beginning.

Measured cadmium concentrations on day 0 in both sediment and seawater were therefore used in all statistical analyses. In contrast, the concentration of cadmium in interstitial water decreased by 50% or more during a bioassay designed to determine the LC_{50} based on the concentration of cadmium in interstitial water. The LC_{50} and statistical analyses were therefore calculated separately for measured cadmium concentrations in interstitial water at both the beginning and end of this experiment.

Per cent total solids (TS) and per cent total volatile solids (TVS) were determined for control sediment and for control sediment that was enriched by the addition of sewage sludge or fine material from natural sediment. TS was calculated as the per cent of the initial wet weight of a sample remaining after drying at 105°C for 16 h (Environmental Protection Agency, 1979). TVS was calculated as the per cent weight loss of the dried sample after it was heated at 550°C for 1 h (Environmental Protection Agency, 1979).

Conditions in the bioassay beakers remained aerobic during these experiments. Sediment redox potential (E_h), measured by a Pt/calomel electrode inserted 1 cm beneath the sediment surface, ranged from +275 to +462 mV relative to the hydrogen electrode. Dissolved oxygen in overlying seawater, measured by a YSI Model 57 DO meter, ranged from 9·3 to 9·9 mg liter^{-1}.

Experimental design

Four experiments using *R. abronius* bioassays were conducted.

I. Cadmium toxicity in sediment

Toxic concentrations of cadmium were determined by 10-day bioassays of sediment containing different total concentrations of cadmium. Data from one range-finding and one definitive bioassay were pooled for statistical analysis. The cadmium LC_{50}, based on the lethal concentration at which 50% of the amphipods did not survive, and EC_{50}, based on the effective concentration at which 50% of the amphipods were not able to rebury at the end of the bioassay, were calculated by probit analysis. The per cent of amphipods responding was regressed against the logarithm of the cadmium concentration to determine the LC_{50} and EC_{50} (Finney, 1971; Statistical Analysis System, 1982). Emergence data are depicted graphically to show the temporal pattern of emergence of

amphipods from sediment during the bioassay. The statistical significance of differences in the mean number of emerged amphipods after 10 days of exposure was tested by ANOVA and Student–Neuman–Keul's multiple range test (Sokal & Rohlf, 1969).

II. Cadmium toxicity in seawater

The 96 h LC_{50} and EC_{50} of cadmium in seawater were determined for *R. abronius* in bioassays without the presence of sediment. Data from two range-finding bioassays and one definitive bioassay were pooled and analyzed as described for Experiment I. Emergence data could not be collected because there was no sediment.

III. Toxicity of interstitial and particle-bound cadmium

Data to determine the relative contributions to toxicity of particle-bound and interstitial cadmium were obtained by repeating Experiment I for an exposure of 4 rather than 10 days. Cadmium concentrations were measured in both the total sediment and in the interstitial water. The 96 h LC_{50} of cadmium in interstitial water was then compared to the 96 h LC_{50} of cadmium in seawater (Experiment II).

Experiment III tested the null hypothesis that there is no difference between the 96 h LC_{50} of cadmium in seawater and the 96 h LC_{50} of cadmium in interstitial water in bioassays where cadmium was added to sediment. If this null hypothesis cannot be rejected, the results would indicate that sediment toxicity can be attributed to cadmium dissolved in interstitial water. If the null hypothesis can be rejected, two alternative hypotheses could be considered. First, a direct toxic effect of particle-bound cadmium would be indicated if the LC_{50} of cadmium in seawater is significantly greater than the LC_{50} in interstitial water. Secondly, if the LC_{50} of cadmium in seawater is significantly less than in interstitial water, this would indicate that the presence of sediment mitigates the effects of cadmium through either biological or chemical factors. Regardless of the results of the statistical comparisons, if the 96 h LC_{50}s of cadmium in seawater and in interstitial water are similar, the dominant toxic effect must be exerted by interstitial cadmium because the majority of cadmium in sediment is particle-bound.

IV. Interactions between cadmium, sewage sludge and the fine fraction of natural sediment

Effects of interactions between cadmium, sewage sludge and fine sediment materials on sediment toxicity to *R. abronius* were examined

in a two-way design with three levels of cadmium (0, 8·8, 13·6 mg kg^{-1}, dry weight) and three levels of enrichment (no addition, sludge addition, natural sediment addition). The total volatile solids of the control (no addition) sediment was 1·05% of the dry weight of the sediment. The measured increase in total volatile solids was 0·29% for the Waldport sewage sludge addition and 0·27% for the natural sediment addition. Five replicates were prepared for each of the nine treatments. The statistical significance of interaction between cadmium and sediment enrichments was analyzed by a Model I two-way ANOVA (Sokal & Rohlf, 1969).

RESULTS

I. Cadmium toxicity in sediment

There was an inverse relationship between the concentration of cadmium in sediment and both mean survival and mean reburial in Experiment I (Table 1). Mean survival of controls for the two bioassays of this experiment was 95%. More than 90% of the amphipods survived and reburied in clean sediment at the end of the exposure to concentrations of 3·5 mg kg^{-1} cadmium in sediment or less. LC_{50} for survival and EC_{50} for reburial were 6·9 and 6·5 mg kg^{-1} cadmium, respectively. At sediment concentrations of 8·09, 9·34 and 16·2 mg kg^{-1} cadmium, survival and ability to rebury decreased progressively, 34·0% of the total number of amphipods that survived at these three concentrations were unable to rebury and the mean number of amphipods that emerged from sediment was significantly greater than at all other concentrations.

Amphipods continued to emerge throughout the 10 days of exposure to sediment containing 8·09 and 9·34 mg kg^{-1} cadmium (Fig. 1). At 16·2 mg kg^{-1}, emergence was most rapid during the first 4–6 days and then declined. At the highest concentration (89·6 mg kg^{-1}), emergence was not significantly different from the control, no amphipods emerged after day 4 and all died during the bioassay.

II. Cadmium toxicity in seawater

Mean survival of control amphipods held for 4 days in seawater without sediment was 95% (Table 2). Among control survivors, 8·1% were not

TABLE 1
Experiment I: Survival, Reburial and Emergence of *R. abronius* After 10 Days of Exposure to Cadmium in Sediment
(each treatment included five replicates of 20 amphipods each)

Bioassay number	*Sediment cadmium concentration* ($mg\,kg^{-1}$)		*Mean number of amphipods*		
			Survival	*Reburial*	*Day 10 emergence*[a]
1	Control	0·00	18·8	18·8	0·4 A
2	Control	0·00	19·2	19·2	0·6 A
1		0·02	18·6	18·6	0·6 A
1		0·07	19·0	19·0	0·4 A
1		0·91	18·2	18·2	1·0 A
2		0·98	19·2	19·0	0·6 A
2		1·94	19·2	19·2	0·4 A
2		3·50	18·2	18·2	0·8 A
2		8·09	8·6	7·0	6·4 B
1		9·34	2·4	1·2	11·6 C
2		16·2	0·6	0·4	12·2 C
1		89·6	0	0	1·2 A
Survival LC_{50}		6·9			
95% Fiducial limits		5·6–7·9			
Reburial EC_{50}		6·5			
95% Fiducial limits		5·1–7·4			

[a] Differences between means are highly significant; ANOVA $F = 27·6$, $F.001[11, 48] = 3·61$. Means followed by different letters are significantly different ($p < 0·05$) as determined by Student–Neuman–Keul's multiple range test.

able to rebury in clean sediment. The LC_{50} for survival was 1·61 mg $liter^{-1}$ of cadmium in seawater, while the EC_{50} for reburial was considerably less, 0·55 mg $liter^{-1}$. The per cent of survivors that were not able to rebury increased from <20% at 0·01–0·11 mg $liter^{-1}$ cadmium in seawater to >70% at concentrations above 1 mg $liter^{-1}$.

III. Toxicity of interstitial and particle-bound cadmium

The 4-day LC_{50} of cadmium in sediment for survival and EC_{50} for reburial were 25·9 and 20·8 mg kg^{-1}, respectively (Table 3). These estimates of toxic concentrations are significantly higher for 4-day exposures (Table 3) than for 10-day exposures (Table 1).

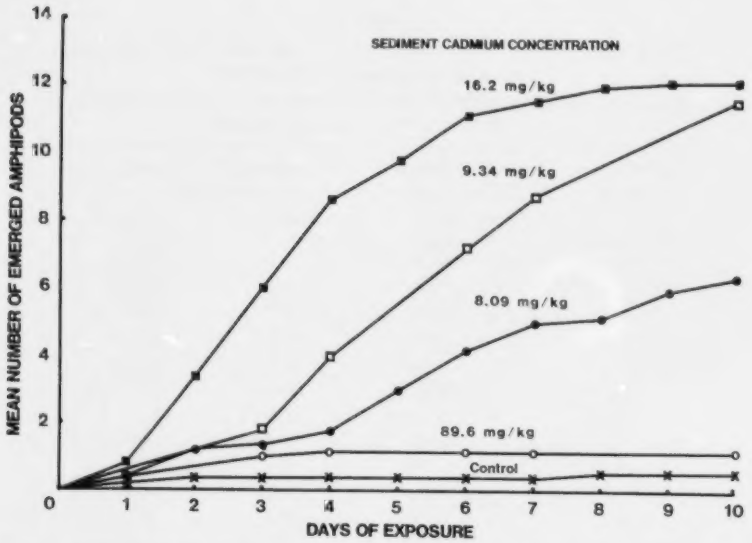

Fig. 1. Mean number of emerged amphipods during 10-day sediment cadmium bioassays. Each treatment included five replicates of 20 amphipods each.

Cadmium in interstitial water decreased to less than 50% of the initial concentration during the 4-day duration of Experiment III (Table 3). LC_{50} estimates were therefore calculated for the concentrations of cadmium in interstitial water on both day 0 and day 4. The LC_{50} for survival was 3·64 mg liter^{-1} for day 0 concentrations and 1·42 mg liter^{-1} for day 4 concentrations. These toxicity estimates for cadmium in interstitial water bracket the LC_{50} of cadmium in seawater (1·61 mg liter^{-1}, Table 2). The null hypothesis of no significant difference between the LC_{50} of cadmium in seawater and the LC_{50} of cadmium in interstitial water on day 4 cannot be rejected, indicating that sediment toxicity can be attributed mainly to cadmium in interstitial water and that any fraction of the toxicity due to sediment particles was too small to be resolved. However, the null hypothesis of no significant difference between the seawater LC_{50} and the day 0 interstitial water LC_{50} can be rejected in favor of our second alternative hypothesis, i.e. the presence of sediment mitigates the effects of cadmium through either biological or chemical factors. Despite the decrease in the concentration of cadmium in interstitial water during this experiment, the statistical

TABLE 2
Experiment II: Survival and Reburial of *R. abronius* After 4 Days of Exposure to Cadmium in Seawater
(sediment was not present during exposure; each treatment included five replicates of 20 amphipods each)

Bioassay number	*Seawater cadmium concentration (mg liter^{-1})*		*Mean number of amphipods*	
			Survival	*Reburial*
1	Control	0·00	18·7	16·7
2	Control	0·00	19·6	18·2
3	Control	0·00	18·6	17·4
1		0·01	17·3	14·7
2		0·06	17·4	15·9
2		0·11	18·4	15·7
1		0·12	18·0	11·5
2		0·23	18·2	13·9
2		0·46	16·2	10·0
1		0·60	16·3	4·8
3		0·63	16·0	9·4
2		0·92	15·2	6·4
1		1·16	10·3	1·3
3		1·24	13·6	3·2
2		1·93	7·6	1·6
3		2·37	5·6	0·6
3		4·85	0·6	0
3		9·46	0	0
1		11·2	0	0
Survival LC_{50}		1·61		
95% Fiducial limits		1·41–1·83		
Reburial EC_{50}		0·55		
95% Fiducial limits		0·32–0·73		

analyses do not allow acceptance of the first alternative hypothesis, i.e. the LC_{50} of cadmium in seawater is significantly greater than the LC_{50} based on interstitial water concentrations. Thus, the results provide no evidence that particle-bound cadmium contributes directly to sediment toxicity.

The cadmium mass balance in the bioassay chambers can be calculated from the 4-day LC_{50} based on total sediment concentration (25·9 mg kg^{-1}) and the corresponding concentration of cadmium in interstitial water at the beginning of the bioassay (3·64 mg liter^{-1}, Table 3).

TABLE 3
Experiment III: Survival, Reburial and Emergence of *R. abronius* After 4 Days of Exposure to Cadmium in Sediment
(each treatment included five replicates of 20 amphipods each)

	Cadmium concentration			*Mean number of amphipods*		
	Sediment (mg kg^{-1}) Day 0	*Interstitial water (mg liter^{-1})*		*Survival*	*Reburial*	*Day 4 emergence*
		Day 0	*Day 4*			
Control	0·00	0·00	0·00	19·6	19·6	0·0
	5·89	0·01	0·01	19·8	19·8	0·2
	11·3	0·47	0·05	19·0	18·2	1·4
	16·7	1·56	0·44	17·0	15·8	2·0
	21·4	2·88	0·88	11·6	7·2	5·4
	30·2	4·51	2·06	7·8	3·8	7·2
Survival LC_{50}	25·9	3·64	1·42			
95% Fiducial limits	24·0–28·3	3·17–4·43	1·16–1·90			
Reburial EC_{50}	20·8	2·43	0·58			
95% Fiducial limits	17·0–25·4	1·44–3·54	0·14–1·56			

Total cadmium in sediment Cd(T):

$$\begin{aligned} Cd(T) &= (\text{sediment } LC_{50})(\text{wet weight of sediment}) \\ &\quad (\%\text{ dry solids of wet sediment}) \\ &= (25{\cdot}9\text{ mg Cd per dry kg sediment}) \\ &\quad (0{\cdot}300\text{ wet kg sediment})(0{\cdot}76) \\ &= 5{\cdot}91\text{ mg Cd} \end{aligned}$$

Total cadmium in interstitial water Cd(IW):

$$\begin{aligned} Cd(IW) &= (\text{IW } LC_{50})(\text{wet weight of sediment}) \\ &\quad (\%\text{ water of wet sediment})(\text{IW density}) \\ &= (3{\cdot}64\text{ mg Cd liter}^{-1})(0{\cdot}300\text{ wet kg sediment})(0{\cdot}24) \\ &\quad (1\text{ liter IW per kg IW}) \\ &= 0{\cdot}26\text{ mg Cd} \end{aligned}$$

Total cadmium bound to particles Cd(P):

$$\begin{aligned} Cd(P) &= Cd(T) - Cd(IW) \\ &= 5{\cdot}65\text{ mg Cd} \end{aligned}$$

The interstitial water at the LC_{50} based on the day 0 concentration therefore contained about 4·4% of the total cadmium in the sediment. The same calculation based on the interstitial water LC_{50} at the end of the bioassay (1·42 mg liter^{-1}) shows that the proportion of cadmium in interstitial water decreased to about 1·7%.

IV. Interactions between cadmium, sewage sludge and the fine fraction of natural sediment

Slight enrichment of the control sediment by either Waldport sewage sludge or the fine fraction of natural sediment reduced the toxicity of cadmium in sediment to *R. abronius* in 10-day bioassays (Table 4). The enrichment treatments in the absence of cadmium did not affect survival, reburial or emergence of amphipods from sediment. In sediment containing 8·8 mg kg^{-1} cadmium, mean survival increased from 2·2 amphipods (11%) for the non-enriched treatment, to 15·6 amphipods (78%) for the natural sediment treatment, to 19·2 amphipods (97%) for

TABLE 4

Experiment IV: Survival, Reburial and Emergence of *R. abronius* After 10 Days of Exposure to Sediment Amended with Cadmium, Waldport Sewage Sludge or Natural Sediment Volatile Solids

(each treatment included five replicates of 20 amphipods each)

Increase in per cent total volatile solids	*Sediment cadmium concentration (mg kg^{-1})*								
	0	*8·8*	*13·6*	*0*	*8·8*	*13·6*	*0*	*8·8*	*13·6*
	Mean survival			*Mean reburial*			*Mean emergence*		
0 (control)	19·8	2·2	0	19·8	0·8	0	0	10·6	9·6
0·27% (natural sediment solids)	19·4	15·6	6·2	19·0	15·0	5·2	0·6	4·2	9·8
0·29% (Waldport sewage sludge)	19·2	19·2	15·4	19·2	19·2	14·8	1·0	0·4	4·6

ANOVA Table for Mean Survival

Source of variation	*df*	*SS*	*MS*	*F*	*p*
Cadmium	2	1 138·53	569·27	201·[illegible]	<0·001
Volatile solids	2	854·80	427·40	151·39	<0·001
Interaction	4	549·07	137·27	48·64	<0·001
Error	36	101·60	2·82		
Total	44	2 644·00			

	Reburial	*Emergence*
Interaction F	61·18 ($p < 0·001$)	12·81 ($p < 0·001$)

the sludge treatment. In sediment containing 13·6 mg kg^{-1} cadmium, no amphipods survived in the non-enriched treatment, 6·2 (31%) survived in the natural sediment treatment and 15·4 (77%) survived in the sludge treatment. Similar results were obtained for the reburial observations (Table 4). Emergence was inversely related to survival and reburial, except that a plateau of about 10 amphipods emerged when survival was 6·2 or less. Effects of interactions between cadmium, sewage sludge and natural sediment enrichment on the survival, emergence and reburial of *R. abronius* were highly significant ($p < 0{\cdot}001$).

Effects of 8·8 and 13·6 mg kg^{-1} cadmium in sediment on survival and reburial were significantly ($p < 0{\cdot}01$) different between the three enrichment treatments. At the same level of volatile solids addition, Waldport sewage sludge was therefore more effective than the fine fraction of natural sediments in reducing the effects of cadmium in sediment on *R. abronius*.

DISCUSSION

Sediment toxicity to *R. abronius* was caused primarily by cadmium dissolved in the interstitial water. The LC_{50} in interstitial water calculated from measured cadmium concentrations at both the beginning and end of the sediment bioassay (3·64 and 1·42 mg $liter^{-1}$, respectively) was not significantly less than the LC_{50} in seawater (1·61 mg $liter^{-1}$). Thus, there was no evidence in favor of the hypothesis that particle-bound cadmium contributed directly to the mortality of *R. abronius*. This conclusion is in agreement with that of Ray *et al.* (1980), who found that the marine polychaete, *Nereis virens*, accumulated cadmium initially added to sediment at rates predicted by cadmium concentrations in overlying seawater. The toxicity of cadmium in sediment to epibenthic freshwater organisms also occurs after partitioning into the aqueous phase (Francis *et al.*, 1984; Schuytema *et al.*, 1984).

Discrimination of the effects of interstitial and particle-bound cadmium is complicated by the difficulty of exposing amphipods to one source and not the other. A potential problem with our experimental design is that toxic effects of particle-bound cadmium might be undetected if the bioavailability of cadmium in interstitial water was less than in seawater. It seems improbable that a contribution to toxicity by the particle-bound cadmium would coincide quantitatively with the

difference in toxicity of dissolved cadmium in seawater and in interstitial water. Rather, the close similarity in the seawater and interstitial LC_{50}s indicates that little, if any, stress was imposed directly by the particle-bound cadmium.

Chronic exposure to particle-bound cadmium may have different toxicological consequences than the acute exposures used in our research. The equilibration time for the partitioning of cadmium between estuarine sediment particles is 1–20 h (Oakley *et al.*, 1981; Davies-Colley, 1982). Although our sediment–cadmium mixtures were prepared 40 h before the beginning of the bioassays, the reduction in the concentration of cadmium in interstitial water during the tests indicates that the system was not in complete equilibrium (Table 3). Less than 2% of the cadmium was in solution at the end of the bioassays. The proportion of cadmium in interstitial water would be even less at equilibrium. Even minor processes of cadmium bioaccumulation directly from particles might be important under such conditions during chronic exposure.

The trophic position of an infaunal species could affect its relative sensitivity to interstitial and particle-bound cadmium. Phoxocephalid amphipods like *R. abronius* are meiofaunal predators (Oliver *et al.*, 1982). Their principal route of accumulation of sediment contaminants may therefore be from interstitial water via the gill. Deposit-feeding infauna may be more susceptible to particle-bound contaminants because of sediment ingestion.

Our observations on the emergence of *R. abronius* support the conclusion of Oakden *et al.* (1984*a*,*b*) that *Rhepoxynius* spp. burrow or swim away from sediment containing cadmium. The rate of emergence increased with cadmium sediment concentrations from 8·09 to 16·2 mg kg^{-1} in 10-day bioassays (Fig. 1) and up to 30·2 mg kg^{-1} in 4-day bioassays (Table 3). At the highest cadmium concentration (89·6 mg kg^{-1}), no animals survived and emergence (6%) was not significantly different from the control (Table 1). These amphipods may have been exposed to a cadmium stress that caused a rapid paralysis or death. At 8·09–16·2 mg kg^{-1} cadmium, most emergence occurred after 2 or 3 days of exposure. In contrast, *R. abronius* emerges within seconds from sediment with very low interstitial water salinity (Swartz, unpublished data). Emergence from cadmium-contaminated sediment may therefore be due to debilitation rather than a sensory recognition of unfavorable conditions. No more than 61% of the amphipods emerged in any cadmium in sediment treatment, although it is possible

that some may have emerged and reburied without being observed. Oakden *et al.* (1984*a*) reported that 44% of amphipods successfully swam away from a very fine sand containing 5·6 mg kg^{-1} cadmium. These laboratory experiments suggest that behavioral avoidance may protect portions of, but not entire, phoxocephalid populations from toxic sediment conditions.

The highly significant interaction between cadmium, sewage sludge and the fine fraction of natural sediment resulted in greatly reduced sediment toxicity to *R. abronius* (Table 4). This result is consistent with the known cadmium-binding capacity of sediment particles (Luoma & Jenne, 1976) and the present finding that interstitial water is the dominant source of cadmium toxicity. Oakden *et al.* (1984*b*) demonstrated that chelation of cadmium by EDTA increased the survival of the haustoriid amphipod *Eohaustorius sencillus* in sediment containing otherwise lethal concentrations of cadmium. Similar results were also obtained by Adams *et al.* (1984), who determined the no-effect concentration of kepone in sediment to the freshwater midge, *Chironomus tentans*, for three sediments with a range in organic carbon content of two orders of magnitude. No-effect concentrations based on total sediment kepone increased in proportion to organic carbon content, but no-effect concentrations based on interstitial water kepone were similar for all sediments.

Our experimental manipulation of the sediment volatile solids content was about 0·28%, well within the seasonal variation of unpolluted sediment at the Yaquina Bay collection site. Sewage disposal on the Palos Verdes Shelf, California, increases sediment volatile solids from a background of 4·0% to over 15% (Swartz *et al.*, 1985*a*). This enrichment greatly reduces the toxicity that might be expected on the basis of total sediment contaminant concentrations. The Los Angeles County Sanitation District collected the phoxocephalid, *Heterophoxus occulatus*, at sites on the shelf, where cadmium concentrations were as high as 19 mg kg^{-1} (cited in Oakden *et al.* (1984*a*)). In laboratory bioassays similar to those used in the present study, sediment collected within 3 km of the sewage outfall on the shelf were acutely toxic (Swartz *et al.*, 1985*a*). However, 77% of *R. abronius* survived 10-day exposures to shelf sediment containing 32 mg kg^{-1} cadmium, 5·6 mg kg^{-1} DDE and high concentrations of many other metals, hydrocarbon and synthetic organic compounds.

Slight enrichment of sediment by sewage sludge was significantly more effective than the fine fraction of natural sediment in binding cadmium

and reducing toxicity (Table 4). The binding capacity of these materials appears to be determined qualitatively by their organic and inorganic composition and cannot be predicted quantitatively by total volatile solids or other measures of organic enrichment. Our results should not be misconstrued. Sewage sludge contains toxic chemicals and is sometimes toxic, by itself, at concentrations used in the present experiments (Swartz *et al.*, 1984). Sewage disposal is a cause, not the solution, of marine metal pollution.

The interaction between cadmium and the geochemical characteristics of sediment precludes a general sediment quality criterion based on total cadmium concentrations. The 10-day LC_{50} for fine sands with low organic content is 6·9 mg kg^{-1}. The highest concentration for which there is no evidence of acute toxicity is about 2 mg kg^{-1} (Table 1). Estimates of acutely toxic concentrations in clean, fine sands can be conservatively extrapolated to more enriched sediment. However, these values are definitely not proven 'safe' concentrations because chronic toxicity and effects on populations were not examined in our research.

Knowledge of the relative tolerance of different organisms to marine pollution is important in the selection of bioassay species. Infaunal phoxocephalid amphipods (including *R. abronius*) have been used recently in a number of laboratory experiments to determine the toxicity of marine sediment (Swartz *et al.*, 1979, 1982, 1984, 1985*a*,*b*; Oakden *et al.*, 1984*a*,*b*). Although few interspecific comparisons have been conducted, *R. abronius* has a relatively high sensitivity to sediment collected from contaminated field sites (Swartz *et al.*, 1979; Chapman & Long, 1983; Chapman, in press). If *R. abronius* is unduly sensitive, regulatory decisions based on phoxocephalid bioassays may be overly protective. Our determination of the acute toxicity of cadmium in seawater to *R. abronius* permits comparison with the cadmium tolerance of many other marine species. Eisler (1971) determined the 96 h LC_{50} for cadmium in 20 ppt seawater for 13 marine species, including crustaceans, molluscs, echinoderms, polychaetes and fish. The LC_{50}s ranged from 0·32 to 55 mg liter^{-1}. The 96 h LC_{50} was less than 1 mg liter^{-1} for four species: sand shrimp, *Crangon septemspinosa*; hermit crabs, *Pagurus longicarpus*; grass shrimp, *Palaemonetes vulgaris*; and starfish, *Asterias forbesi*. The 96 h LC_{50} for cadmium in 25 ppt seawater to *R. abronius* is 1·61 mg liter^{-1} (Table 2). The toxicity of cadmium in seawater is inversely related to salinity because of the complexation of free cadmium ions by chloride ions (Sunda *et al.*, 1978). However, the

approximate 20% decrease in the concentration of free cadmium ions that is caused by a salinity change from 20 ppt seawater (used by Eisler, 1971) to 25 ppt (present study) is not sufficient to alter the conclusion that *R. abronius* is no more sensitive to cadmium than several other abundant and ecologically important benthic invertebrates. It is appropriate therefore to continue to use this amphipod in bioassays of sediment contamination.

The absence of sediment can affect the behavior of benthic animals in the laboratory and possibly increase sensitivity to contaminants (Pesch & Morgan, 1978). Control survival in the 4-day seawater bioassays averaged 95%, certainly an acceptable level (Table 2). However, 8·1% of the seawater control survivors were unable to rebury in sediment at the end of the experiments. In contrast, all control survivors in bioassays conducted with sediment were able to rebury (Tables 1 and 3). The interaction that may have occurred between the toxicity of cadmium in seawater and sublethal stresses caused by the absence of sediment may have artificially decreased the 4-day LC_{50} for cadmium in seawater. It is unlikely that this possible interaction would be sufficient to change our conclusions about the relative sensitivity of *R. abronius* to cadmium or the contribution of interstitial cadmium to sediment toxicity.

ACKNOWLEDGMENTS

We thank Robert Barrick, Bori Olla and Paul Kemp for reviewing the manuscript, and Jill Jones, Faith Cole and Kathy Sercu for assistance and support.

REFERENCES

Adams, W. J., Kimerle, R. A. & Mosher, R. G. (1984). Aquatic safety assessment of chemicals sorbed to sediments. In: *Aquatic toxicology and hazard assessment: seventh symposium* (Cardwell, R. D., Purdy, R. & Bahner, R. C. (Eds)), American Society for Testing and Materials, Philadelphia, STP 854, 429–53.

Bryan, G. W. (1976). Heavy metal contamination in the sea. In: *Marine pollution* (Johnston, R. (Ed.)), Academic Press, Inc., New York, 185–302.

Chapman, P. M. (in press). Sediment bioassay tests provide toxicity data necessary for assessment and regulation. *Proceedings 11th Annual Aquatic Toxicity Workshop*, 13–15 November 1984, Vancouver, BC.

Chapman, P. M. & Long, E. R. (1983). The use of bioassays as part of a comprehensive approach to marine pollution assessment. *Mar. Poll. Bull.*, **14**, 81–4.

Davies-Colley, R. J. (1982). Estuarine sediment controls on trace metal distributions. PhD Thesis, Oregon State University, Corvallis, Oregon.

Davies-Colley, R. J., Nelson, P. O. & Williamson, K. J. (1984). Copper and cadmium uptake by estuarine sedimentary phases. *Environ. Sci. Technol.*, **18**, 491–9.

Eisler, R. (1971). Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. *J. Fish. Res. Bd. Canada*, **28**, 1225–34.

Environmental Protection Agency (1979). *Methods for chemical analysis of water and wastes*. US Environmental Protection Agency, Cincinnati, Ohio, EPA-600/4-79-20.

Finney, D. J. (1971). *Probit analysis*, Cambridge University Press, Cambridge, UK.

Francis, P. C., Birge, W. J. & Black, J. A. (1984). Effects of cadmium-enriched sediment on fish and amphibian embryo-larval stages. *Ecotoxicol. Environ. Safety*, **8**, 378–87.

Graney, R. L. Jr, Cherry, D. S. & Cairns, J. Jr (1984). The influence of substrate, pH, diet, and temperature upon cadmium uptake in the Asiatic clam (*Corbicula fluminea*) in laboratory artificial streams. *Wat. Res.*, **18**, 833–42.

Hardy, J. T., Schmidt, R. L. & Apts, C. W. (1981). Marine sediment and interstitial water: effects on bioavailability of cadmium to gills of the clam *Protothaca staminea*. *Bull. Environ. Contam. Toxicol.*, **27**, 798–805.

Helz, G. R., Huggett, R. J. & Hill, J. M. (1975). Behavior of Mn, Fe, Cu, Zn, Cd and Pb discharged from a wastewater treatment plant into an estuarine environment. *Wat. Res.*, **9**, 631–6.

Hershelman, G. P., Schafer, H. A., Jan, T.-K. & Young, D. R. (1981). Metals in marine sediments near a large California municipal outfall. *Mar. Poll. Bull.*, **12**, 131–4.

Luoma, S. N. & Jenne, E. A. (1976). Factors affecting the availability of sediment-bound cadmium to the estuarine, deposit-feeding clam, *Macoma balthica*. In: *Radioecology and energy resources: proceedings of the fourth national symposium on radioecology* (Cushing, C. E. (Ed.)), Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pennsylvania, 283–91.

Oakden, J. M., Oliver, J. S. & Flegal, A. R. (1984*a*). Behavioral responses of a phoxocephalid amphipod to organic enrichment and trace metals in sediment. *Mar. Ecol. Prog. Ser.*, **14**, 253–7.

Oakden, J. M., Oliver, J. S. & Flegal, A. R. (1984*b*). EDTA chelation and zinc antagonism with cadmium in sediment: effects on the behavior and mortality of two infaunal amphipods. *Mar. Biology*, **84**, 125–30.

Oakley, S. M., Nelson, P. O. & Williamson, K. J. (1981). Model of trace-metal partitioning in marine sediments. *Environ. Sci. Technol.*, **15**, 474–80.

Oliver, J. S., Oakden, J. M. & Slattery, P. N. (1982). Phoxocephalid amphipod

crustaceans as predators on larvae and juveniles in marine soft-bottom communities. *Mar. Ecol. Prog. Ser.*, **7**, 179–84.

Pesch, C. E. & Morgan, D. (1978). Influence of sediment in copper toxicity tests with the polychaete *Neanthes arenaceodentata*. *Wat. Res.*, **12**, 747–51.

Ray, S., McLeese, D. & Pezzack, D. (1980). Accumulation of cadmium by *Nereis virens*. *Arch. Environ. Contam. Toxicol.*, **9**, 1–8.

Riffaldi, R., Levi-Minza, R., Saviozzi, A. & Tropea, M. (1983). Sorption and release of cadmium by some sewage sludges. *J. Environ. Qual.*, **12**, 253–6.

Schuytema, G. S., Nelson, P. O., Maleug, K. W., Nebeker, A. V., Krawczyk, D. F., Ratcliff, A. K. & Gakstatter, J. H. (1984). Toxicity of cadmium in water and sediment slurries to *Daphnia magna*. *Environ. Toxicol. Chem.*, **3**, 293–308.

Sokal, R. R. & Rohlf, F. J. (1969). *Biometry*, W. H. Freeman, San Francisco.

Statistical Analysis System (1982). *SAS user's guide: statistics*, SAS Institute, Inc., Cary, North Carolina.

Sunda, W. G., Engel, D. W. & Thuotte, R. M. (1978). Effect of chemical speciation on toxicity of cadmium to grass shrimp, *Palaemonetes pugio*: importance of free cadmium ion. *Environ. Sci. Technol.*, **12**, 409–13.

Swartz, R. C., DeBen, W. A. & Cole, F. A. (1979). A bioassay for the toxicity of sediment to marine macrobenthos. *J. Wat. Poll. Control. Fed.*, **51**, 944–50.

Swartz, R. C., DeBen, W. A., Sercu, K. A. & Lamberson, J. O. (1982). Sediment toxicity and the distribution of amphipods in Commencement Bay, Washington, USA. *Mar. Poll. Bull.*, **13**, 359–64.

Swartz, R. C., Schults, D. W., Ditsworth, G. R. & DeBen, W. A. (1984). Toxicity of sewage sludge to *Rhepoxynius abronius*, a marine benthic amphipod. *Arch. Environ. Contam. Toxicol.*, **13**, 207–16.

Swartz, R. C., Schults, D. W., Ditsworth, G. R., DeBen, W. A. & Cole, F. A. (1985*a*). Sediment toxicity, contamination and macrobenthic communities near a large sewage outfall. In: *Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems* (Boyle, T. P. (Ed.)), American Society for Testing and Materials, Philadelphia, STP 865, 152–75.

Swartz, R. C., DeBen, W. A., Jones, J. K. P., Lamberson, J. O. & Cole, F. A. (1985*b*). Phoxocephalid amphipod bioassay for marine sediment toxicity. In: *Aquatic toxicology and hazard assessment: seventh symposium* (Cardwell, R. D., Purdy, R. & Bahner, R. C. (Eds)), American Society for Testing and Materials, Philadelphia, STP 854, 284–307.

Thomson, E. A., Luoma, S. N., Johansson, C. E. & Cain, D. J. (1984). Comparison of sediments and organisms in identifying sources of biologically available trace metal contamination. *Wat. Res.*, **18**, 755–65.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.